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STUDIES ON AUDITORY AND VESTIBULAR END ORGANS AND BRAIN STEM NUCLEI

Harlow W. Ades, Principal Investigator

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STUDIES ON AUDITORY AND VESTIBULAR END ORGANS AND BRAIN STEM NUCLEI

Harlow W. Ades, Principal Investigator

This research program has been supported by the National Aeronautics & Space Administration for a period covering over nine years since the Principal Investigator came to the University of Illinois from the U. S. Naval School of Aviation Medicine (now the U. S. Naval Aerospace Medical Institute), Pensacola, Florida. The research has encompassed a broad spectrum of effort in the field of noise exposure and its effects. It has also contributed much to the understanding of the vestibular system as it affected the performance of the astronauts in the space program. The research has been a pioneering effort in the development of electron microscopy (both transmission and later scanning electron microscopy), and has shown otherwise unknown structures and formations in the anatomy of the inner ear and vestibular systems.

The NASA Technical Officers appointed as monitors of this grant over the years were as follows:

1965 - 1969: Walton L. Jones, NASA HQ

1970 - 1971: Randall Chambers and Phil Edge - Langley

1971 - 1973: David Winter, Ames Research Center

1973 - 1974: William Mehler, Ames Research Center

The Principal Investigator has served on numerous panels and committees for NASA and for many years was a member of the NASA Research Advisory Committee on Advanced Research and Technology (OART). In this regard he was appointed a member of the Italian National Research Council in 1968 and served until 1971. The Council met in Milano, Italy, and reviewed research being done by Dr. Torquato Gualtierotti at the Space Biology Laboratory in that city. Professor Ades spent three years as a member of the University of Illinois' Task Force on Noise in conjunction with the Illinois Pollution Control Board. As such he was instrumental in achieving the passing of the first Illinois statutes on the regulation of stationary noise. He is presently a member of

the Executive Council of National Academy of Sciences' Committee on Hearing and Bioacoustics (CHABA) after having served many years on subcommittees to CHABA on noise problems. Research results have been disseminated by the participation of the Principal Investigator and other members of the laboratory at many scientific meetings where papers have been presented (refs. 2, 5, 6, 9, 13, 16, 18, 27), including participation in each of the NASA Symposia (refs. 3, 4, 10, 15) which were held.

There have been joint efforts in this project involving laboratories that have mutual interests in the research, and in furthering the knowledge in the field. Some of them include The University of Gothenburg and the University of Uppsala in Sweden where Professor Dr. Hans Engström has participated extensively in the electron microscopy aspect of the program. Numerous joint authorships of publications in the forms of Books edited (refs. 19,25), book written (ref. 1), Chapters (refs. 18, 20, 21, 23, 24), and articles (refs. 7, 14, 17) have added to the literature in this field. Dr. W. D. Neff at Indiana University has had a joint project with Illinois in this same regard (refs. 16, 24, 28); Loyola University of Chicago with Dr. Terry Dolan (refs. 16, 28); Emory University, Yerkes Primate Center, with Dr. Geoffrey Bourne; and Naval Aviation Medical Institute with Dr. Ashton Graybiel.

As a result of these joint efforts, the Bioacoustics Research Laboratory, University of Illinois, has been host to quite a number of investigators who have spent anywhere from a few weeks to a year and a half in residence here, developing techniques and taking advantage of the particularly unique advantages available. Some of these included: Goran Bredberg, M. D. (University of Gothenburg and University of Uppsala) who spent one and a half years here (refs. 2, 5, 13, 14, 16, 17, 20, 22, 28); Dr. Henrik H. Lindeman (University of Gothenburg and University of Uppsala) who also spent one and a half years here (refs. 3, 14, 15, 17, 22); Ms. Berit Engström, (University of Uppsala) for one year and several shorter periods (ref. 25). One of our Illinois graduate students, Jerome Sugar spent three months studying in Dr. Engström's laboratory

in Uppsala. A direct result of his association with the two laboratories was his contribution of an article, "Stria Vascularis," which appeared in Inner Ear Studies (Ades and Engström, editors). Dr. Sugar was in his last year at Medical School at the time and is presently doing his residency with Dr. Harold Schuknecht at Massachusetts General Hospital in Boston. Another one of our graduate students, Charles W. Stockwell (refs. 10, 11, 12), completed his Ph.D. with Professor Ades as his advisor, was accepted in the U. S. Army and received orders to Naval Aerospace Medical Institute, Pensacola, where he worked for three years with a team there on noise effects and vestibular effects. He is now on the faculty at The Ohio State University in Columbus.

The research under this grant has furthered the knowledge and added expertise to the field of inner ear morphology, and the effects of noise stimulation on the organ of Corti; by its contribution to the training of graduate students who have gone into the field as a direct result of their courses and participation in the research programs offered by the Bioacoustics Research Laboratory. The Principal Investigator has appointments in three different departments at the University of Illinois (Electrical Engineering, Physiology & Biophysics, and Psychology),and through such affiliation, draws the special types of students who can make a definite contribution through their doctoral programs and research. He has been thesis advisor (and served on doctoral committees) to students from other departments such as Speech and Hearing, Biochemistry, and the School of Basic Medical Sciences. This means that each student has spent a period of concentrated study - anywhere from two to four years - on his graduate courses and the research that ultimately results in the material for the doctoral thesis and other publications (refs. 8, 11, 12, 14, 15, 19, 26, 27). During this time, as each student has completed the prescribed curriculum and written a report of the work, acknowledgment has been made to NASA for the support received through the grant to Professor Ades (NASA NGL 14 005 074).

REFERENCES

Major publications covering period October 1965 - December 1974, with selected sample abstracts illustrating the trend the research has taken. Asterisks indicate the articles that have abstracts or summaries included.

1. Engström, H., Ades, H. W. and Andersson, A., Structural pattern of the Organ of Corti, 172 pages, Almqvist & Wiksell/Stockholm, Sweden, 1966.

2. Engström, H., Ades, H. W., and Bredberg, G., Cytoarchitecture of the mammalian organ of Corti. Presented at Second Extra-ordinary Congress, Kyoto, Japan. International Audiology, Vol. V., No. 2, pp. 86-90, 1966.

3. Engström, H., Lindeman, H. H., and Ades, H. W., Anatomical features of the auricular sensory organs. Second Symposium on The Role of the Vestibular Organs in Space Exploration (Moffett Field, Calif.) NASA SP-115, Jan. 1966, pp 33-46.

4. Ades, H. W. and Engström, H., Needs, plans, and identification of the pathologic effects of prolonged exposure to weightlessness on the vestibular Organs in Space Exploration (Moffett Field, Calif.) NASA SP-115, pp 257-262, 1966.

5. Bredberg, G., Engström, H., and Ades, H. W., Cellular pattern of the human cochlea. Excerpta Medica International Congress Series No. 113, Proceedings of the VIIIth International Congress of Otorhinolaryngology. Tokyo, Japan. pp. 494-496, 1965.

6. Ades, H. W., Cortical Projections of the Organ of Corti. Proceedings, Symposium: The Inner Ear with Special Reference to Electron Microscopy. London: Univ. of London, The Institute of Laryngology and Otology, 1966.

* 7. Kellerhals, B., Engström, H., and Ades, H. W., Die Morphologie des Ganglion spirale cochleae. Acta Oto-Laryngologica Suppl. 226, 1967.

* 8. Capps, M. J. and Ades, H. W., Auditory Frequency Discrimination after Transection of the Olivo-Cochlear Bundle in Squirrel Monkeys. Exper. Neurol. 21, 147-158, 1968.

9. Engstrom, H., Ades, H. W., Andersson, A., Bredberg, G. and Lindeman, H. H., Cytoarchitecture of the Normal and Pathologically Altered Inner Ear. Symposium entitled: Gegenwartiger Stand der Cochleaforschung, (Prof. H. Jakobi, Ed.) Hals=, Nasen= und Ohrenheilkunde, Heft 20, pp. 35-53, 1968.

10. Ades, H. W., Stockwell, C. W., Poche, Jr., L. B., and Engström, H., Patterns of Hair Cell Loss in Guinea Pigs after Intense Stimulation by Sinusoidal Sound. In, Proceedings, Fourth Symposium on The Role of the Vestibular Organs in Space Exploration, 24-26 Sept 1968, Pensacola, Fla. NASA SP-187, pp. 285-298, 1970.

* 11. Poche, Jr., L. B., Stockwell, C. W., and Ades, H. W., Cochlear Hair Cell Damage in Guinea Pigs after Exposure to Impulse Noise. J. Acous. Soc. Amer. 46:4 (Part 2), pp. 947-951, 1969.

* 12. Stockwell, C. W., Ades, H. W., and Engström, H., Patterns of Hair Cell Damage After Intense Auditory Stimulation. Annals of Otol. Rhinol. and Laryngol., 78:6, pp. 1144-1169, 1969.

* 13. Engström, H., Ades, H. W., and Bredberg, G., Normal Structure of the Organ of Corti and the Effect of Noise-Induced Cochlear Damage. In, Sensorineural Hearing Loss (G.E.W. Wolstenholme and J, Knight, Eds.), A ciba Foundation Symposium, J. & A. Churchill, London, pp. 127-156, 1970.

* 14. Bredberg, G., Lindeman, H. H., Ades, H. W., West, R., and Engström, H., Scanning Electron Microscopy of the Organ of Corti. Science, 170, pp. 861-863, 1970.

15. Lindeman, H. H., Ades, H. W., and West, R., Scanning Electron Microscopy of the Vestibular End Organs, In, Proceedings Fifth Symposium on The Role of the Vestibular Organs in Space Exploration. 19-21 Aug 1970, Pensacola, Fla., NASA SP-314, pp. 145-155, 1973.

16. Dolan, T. R., Bredberg, G., Ades, H. W., Neff, W. D., The Behavioral and Histological Effects of Auditory Overexposure on the Cat. Presented by Dr. Dolan at the 80th Meeting, Acoustical Society of America., Houston, Texas, Nov. 1970.

* 17. Lindeman, H. H., Ades, H. W., Bredberg, G., and Engström, H., The Sensory Hairs and the Tectorial Membrane in the Development of the Cat's Organ of Corti, A Scanning Microscopic Study. Acta Otolaryng. (Stockh.) 72:229-242, 1971.

18. Ades, H. W., Physiological Hazards of Extended Space Flights. In, Biotechnology, NASA SP-205, pp 193-198, 1971.

* 19. Ades, H. W., and Engström, H.: (EDITORS) INNER EAR STUDIES. Acta Oto-Laryngologica, Suppl 301, 126 pages, Almqvist & Wiksell, Uppsala, Sweden, 1972.

* 20. Bredberg, G., Ades, H. W., and Engström, H., Scanning Electron Microscopy of the Normal and Pathologically Altered Organ of Corti. In, Inner Ear Studies (H. W. Ades and H. Engström, eds.) Almqvist & Wiksell, Uppsala, Sweden, Acta Otolaryngol. Suppl. 301, 3-48, 1972.

21. Engström, H., Bergstrom, B., and Ades, H. W., Macula Utriculi and Macula Sacculi in the Squirrel Monkey. In, Inner Ear Studies (H. W. Ades and H. Engström, eds.). Almqvist & Wiksell, Uppsala, Sweden, Acta Otolaryngol. Suppl. 201, 75-126, 1972.

* 22. Lindeman, H. H. and Bredberg, G., Scanning Electron Microscopy of the Organ of Corti after Intense Auditory Stimulation: Effects on Stereocilia and Cuticular Surface of Hair Cells. Arch. klin. exp. Ohr., Nas.-u. Kehlk. Heilk. 203, 1-15, 1972. (Springer-Verlag, Berlin).

23. Ades, H. W., Auditory System, Chapter 14, In, Bioastronautics Data Book (J. F. Parker, Jr., and V. R. West, eds.), NASA SP-3006, second edition, 667-691, 1973.

24. Ades, H. W. and Engstrom, H., Anatomy of the Inner Ear, Chapter 5, In, Handbook of Sensory Physiology, V/1, Auditory System (W. D. Neff and W. D. Keidel, eds.). Springer-Verlag (Berlin), 125-158, 1974.

25. Ades, H. W. and Engstrom, H., Editors of INNER EAR STUDIES II, Acta Otolaryngologica Supplement 319, Almqvist & Wiksell, Uppsala, Sweden, 66 pages, 1974.

26. Ades, H. W., Trahiotis, C., Kokko-Cunningham, A., and Averbuch, A., Comparison of Hearing Thresholds and Morphological Changes in the Chinchilla after Exposure to 4 KHz Tones. Acta Otolaryng. 78:192-206, 1974. (Reprint appended)

27. Seaton, W. H. and Trahiotis, C., "A Comparison of Critical Ratios and Critical Bands in the Monaural Chinchilla." J. Acoust. Soc. Amer. 57:1, 193-199, 1975.

28. Dolan, T. R., Ades, H. W., Bredberg, G., and W. D. Neff, "Inner Ear Damage and Hearing Loss after Exposure to Tones of High Intensity." (In press: Acta Otolaryngologica; manuscript copy enclosed).

Manuscript copies of these two articles are appended.

SUMMARY

As stated briefly in the opening paragraph of this report, the support of this research project by NASA has enabled the Principal Investigator and his collaborators to add much to the field of knowledge of the organ of Corti and of the vestibular epithelia. It is stated much more to the point in the Introduction of the article authored by G. Bredberg, H. W. Ades, and H. Engström, "Scanning Electron Microscopy of the Normal and Pathologically Altered Organ of Corti," which appears in INNER EAR STUDIES. The following somewhat paraphrases that portion.

The authors have published a series of articles over a period of several years dealing with the morphology of the inner ear of animals and man. Many of those have been devoted to a systematic mapping of the sensory cells and nerve elements of normal and pathologically altered cochlear and vestibular epithelia. They were predicated initially on the idea of portraying properly the cell destruction caused by noise and ototoxic antibiotics. It was considered further that they would gain by the application of electron microscopic techniques. Transmission electron microscopy, while yielding illuminating insights on certain qualitative factors, did not give a quantitative estimate of the sensory cell population in normal or damaged cochleas, as it was known it would not. This left a need for a method of quantitating cells throughout the sensory organ, which in turn led to the development of the surface specimen method by which each sensory cell of the organ of Corti can be examined in situ and the entire organ mapped accordingly by light/phase contrast microscopy.

During the further development of the surface specimen method and its application to problems of noise exposure, the technique of scanning electron microscopy became available to us, and has proven to enjoy certain advantages over both light/phase contrast microscopy and transmission electron microscopy, though it does not supplant either. What it does in part is to bridge the wide gap between light microscopy and transmission electron microscopy. It is an additional adjunct to the study of the inner ear, and only by the judicious combinations of methods and their convergence on the same problems can progress be firmly made. Thus,

conventional transmission electron microscopy has been used widely and has contributed greatly to the knowledge of inner ear morphology. Likewise, light/phase contrast microscopy has clarified, both before and since, many interesting aspects of the morphology of bot cochlear and vestibular portions of the inner ear. Scanning electron microscopy has already had a considerable impact on this research, illuminating structures which were previously seen dimly or not at all. It does so by showing true three-dimensional pictures with a great depth of field and good resolution. It has added a new dimension to cochlear and vestibular morphology, which, while mainly in the realm of surface features, is applicable to a more limited degree to subsurface structures as well.

Most of the pictures presented in this paper represent some aspect of the normal morphology of the organ of Corti. They illustrate the three-dimensional structure in a new way that was made possible by the scanning electron microscope. There are a few figures, mainly transmission electron micrographs, which show the interrelationship and interdependence of transmission electron microscopy and scanning electron microscopy, or illustrate in a different way what is seen in a scanning picture. There are some few figures which are taken from pathological cochleas, or fetal cochleas, and are included to provide illustrations of a few things that can be done with scanning electron microscopy, and to indicate a few obvious areas of future research. This presentation in no way claims to give a complete picture of the morphology of the organ of Corti.

On this note, it should be pointed out that this laboratory is now operating on a very limited budget, and although there are several publications, either in press or in manuscript form ready to be submitted to journals, these will be acknowledged under the present grant from NASA, NGR 14 005 221 which covers the period of one year from January 1975 in the amount of $34,965. We are hoping to have continued support from NASA to complete results on data that have accumulated through the past several years of research.

Richard R. ALMON, Ph.D. 1971: "The Effect of Nerve Growth Factor upon Axoplasmic Transport in Sympathetic Neurons of the Mouse."

Roger W. WEST, Ph.D. 1970: "Electron Microscopy of Normal and Degenerated Ground Squirrel Retina." (See refs. 14 and 15) Thesis summary:

The synaptic organization of the inner plexiform layer of ground squirrel retina was studied using electron microscopy and related to its ganglion cell receptive fields. Other studies have shown that species with a majority of ganglion cells that respond optimally only to complex stimuli have more amacrine-amacrine and serial amacrine synapses and fewer bipolar-ganglion synapses than species with a majority of ganglion cells that respond well to simple stimuli. The present study found that ground squirrel retina, which has about equal numbers of both types of ganglion cells, also has numbers of synapses per unit area between those found in species with a majority of one or the other type of ganglion cell.

It has been reported that in ground squirrel all ganglion cells that respond optimally to complex stimuli project their fibers to the superior colliculus and all ganglion cells that respond well to simple stimuli project their fibers to the lateral geniculate. This made it possible to compare the synaptic inputs of ganglion cells isolated with respect to receptive field type. One group of ground squirrels was lesioned in the lateral geniculate and another group in the superior colliculus. Then, after allowing time for ganglion cell degeneration, the synaptic input of isolated populations of ganglion cells which responded best to complex or simple stimuli was studied.

The results indicated that both types of ganglion cells received nearly equal proportions of bipolar synapses but that ganglion cells which responded best to complex stimuli received at least twice as many amacrine synapses as did ganglion cells that responded well to simple stimuli.

1928

Inner Ear Damage and Hearing Loss after Exposure to Tones of High Intensity[1]

by

T. R. Dolan[2], H. W. Ades, G. Bredberg[3], and W. D. Neff

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[1] The research reported in this paper was supported by grants from the National Aeronautics and Space Administration, NGL 14-005-074 (Ades) and NGL 15-003-077 (Neff).

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Abstract

Experimental animals (cats) were exposed to tones of 125, 1000, 2000, and 4000 Hz at sound pressure levels in the range 120 to 157.5 dB, and for durations of one hour (1000, 2000, 4000 Hz) or four hours (125 Hz). Pure tone audiograms were obtained for each animal before and after exposure. Post-exposure tests were continued until complete recovery of hearing had occurred or until a stable permanent threshold shift had been measured. Cochleas of animals were examined by phase-contrast microscopy; condition of all hair cells was recorded. Extent of inner-ear damage and range of frequencies for which hearing loss occurred increased as exposure tone was decreased in frequency. For example, exposure to 4000 Hz produced damage in a restricted region of the cochlea and hearing loss for a relatively narrow range of frequencies; exposure to 125 Hz produced wide-spread inner ear damage and hearing loss throughout the frequency range 125 to 6000 Hz.

INTRODUCTION

Since the early days of experimental studies of hearing, investigators have used tones or noise of high intensity to stimulate the ear of an experimental animal and, thus, produce damage to structures and change in function of the inner ear. In a review of the literature " on the problem of stimulation deafness", Kemp in 1935 (—) cited 44 published reports of clinical and experimental investigations. The earliest clinical study mentioned that went beyond casual observations was published in 1890 (Habermann); the earliest experimental study, in 1907 (Wittmaack).

With the development of new techniques of examining inner ear pathology and of measuring inner ear function, important new information has come repeatedly through the use of this old procedure which allows physical characteristics of the exposure stimulus to be correlated with inner ear pathology; stimulus characteristics, with changes in inner ear function; and inner ear pathology, with changes in inner ear function.

Major advances in methods of detecting and mapping inner ear damage have included (in more or less chronological order):

1) improved techniques of sectioning and staining of mammalian cochleas; 2) graphic reconstruction of cochleas from serial sections; 3) surface preparation of organ of Corti and use of phase-contrast microscopy to examine and map all hair cells; 4) examination of selected regions of organ of Corti by transmission electron microscopy; 5) examination of cochlear structures by scanning electron microscopy.

The important methodological advances in studying inner ear function have been: 1) recording electrical responses produced by inner ear elements; 2) use of behavioral methods to obtain accurate measures of auditory discrimination (absolute and differential thresholds).

In the majority of published reports, inner ear damage has been assessed and related to frequency, intensity, duration and complexity of the sounds used to produce damage; or change in inner ear function as measured by electrophysiological or behavioral techniques has been related to physical aspects of the exposure stimulus. There have been a relatively small number of studies in which inner ear damage and inner ear function have been examined in the same animal after controlled exposure to sound stimuli. In the earliest of these experiments, inner ear pathology was assessed by examination of selected serial sections through the cochlea; graphic reconstructions were made according to the method of Guild.

Bredberg and Hunter-Duvar (in press) have recently published a summary of the results of animal experiments and human studies in which changes in hearing have been related to inner ear damage that was produced by exposure to high intensity sounds of by other means, such as surgery or administration of ototoxic drugs.

Through the use of the surface preparation of the cochlea and examination by phase-contrast microscopy, a more precise and complete picture of damage to the organ of Corti can be obtained. In a preliminary report, Dolan, Bredberg, Ades, and Neff (1970) described the results of an experiment in which the hearing of cats was tested before and after exposure to pure tones at high sound pressure levels. After sacrifice, surface preparations of the cochleas were made and all hair cells and adjacent structures were examined by phase-contrast microscopy. Details of this study, including results not available at the time of the preliminary report are given below.

METHODS AND PROCEDURE

The general scheme of the investigation included pre-exposure audiometric testing, controlled exposure to a potentially destructive stimulus, post-exposure audiometric testing, and post-mortem examination of the inner ear.

Prior to initial training of the cats, one cochlea of each animal was destroyed by surgery. Additionally, the pinna of the experimental ear was removed and the surrounding area was reconstructed. Removal of the pinna was advantageous in that

it decreased the variance in intensity of acoustic stimuli at the ear drum due to positional changes of the pinna during behavioral testing. The absence of a pinna also allowed close examination of the ear drum prior to and following exposure, accurate monitoring of acoustic waveforms during exposure, and easy accessibility for cleaning of the external canal.

Exposure

For exposure to a high-intensity sound (tone), each experimental animal was anesthetized with diabutal and positioned in a stereotaxic instrument. Its head was held only by the bite bar. The appropriate stimulus frequency was generated by a low distortion General Radio oscillator. The output of the oscillator was fed through an Altec 1569A amplifier and into an Altec 802 D speaker. The output of the speaker passed through an adapter, consisting of telescoping brass tubes (this allowed for tuning by varying the length of the tube), and finally into a speculum that was inserted into the pinnectomized ear. The sound intensity was monitored through a 1 mm probe tube placed just beyond the orifice of the speculum; the probe was connected to a 1/2 inch Bruel and Kjaer microphone (Model 4134); the output of the microphone was amplified by a cathode follower amplifier (B&K 2615, 2801 power supply) and fed into a General Radio sound vibration analyzer. Sound and distortion data were calibrated with a B&K 4220 pistonphone and probe calibration coupler.

For the data presented in the Results section, frequencies of 125 Hz, 1000 Hz, 2000 Hz, and 4000 Hz were examined. At each frequency, the effects of sound pressure levels were also investigated.

Behavioral testing

Audiograms were obtained by an avoidance conditioning procedure. In order to avoid shock, the experimental animal was trained to cross from one side to the other of a double-grill cage when a series of tone pulses was presented. Each tone pulse had a duration of one second and rise and fall times of 300 msec each. The interval between pulses was one-half second. On any given trial, the tone pulses were presented for a period of ten seconds followed by shock given through the bars that formed the floor, sides, and top of the double grill cage.

Tone pulses were generated by a General Radio 1310-A oscillator, amplified by a McIntosh MC-250 amplifier, gated without respect to phase by an electronic switch (Olson and Ludwig, 1965), and presented via an Altec-Lansing 802 D speaker. The loud-speaker and double grill cage were located in a sound-deadened, double-walled room. Sound generating and control instruments were outside of the room. The experimenter sat at a control panel and could observe the experimental animal through a one-way window.

After an animal had learned to make avoidance responses to tones well above threshold level, the minimum sound pressure level (SPL) to which the animal would respond was determined

at each of the following frequencies: 125, 250, 500, 1000, 2000, 4000, 8000, and 12000 Hz. The general procedure for determining a "threshold" for a given frequency involved the attenuation of the tone by 15 B after each correct response. When a level was reached at which no response was made, the tone was increased by 15 dB and then attenuated in 5dB steps until another failure occurred. This procedure was repeated until the animal reversed his behavior from "responding" to "not responding" in the same 5 dB step twice in succession. Following determination of a "threshold" in this manner, the tone was increased by 50 to 80 dB and the entire procedure repeated. Two "thresholds" were obtained at a single frequency during each test session. Pre-exposure testing was continued until stable audiograms had been obtained, that is, until the range of threshold values at each frequency had decreased to 10 dB or less for several successive tests.

A similar procedure was followed after exposure to a high intensity tone. Audiograms were measured until recovery to normal pre-exposure levels occurred or until the amount of measured threshold shift remained constant for a period of several weeks. The time from exposure until sacrifice varied from two to four months for the animals included in the present report.

Post-mortem anatomical methods

The preparation of the cochleas for post-mortem examination was essentially similar to that described in detail by Engstrom,

Ades, and Andersson (1966), except that the entire organ of Corti was displayed on slides as surface specimens. All hair cells were counted and the results recorded on punch cards for computer analysis. The resultant cochleograms give a picture of the remaining or intact hair cells.

RESULTS

The behavioral data showing amount of hearing loss (PTS, permanent threshold shift) and the anatomical data showing the percent of hair cells missing as a result of the exposure to a tone of high intensity are summarized below for 16 animals. The amount of hearing loss suffered by an animal at a particular frequency is the difference in the median value of the final six measures obtained prior to the exposure and the final six measures prior to post-mortem examination.

Exposure to 4000 Hz

The behavioral and anatomical results for three animals exposed to a 4000 Hz tone are shown in Figure 1. The top half of Figure 1 shows the amount of hearing loss (relative to pre-exposure audiogram) as a function of frequency. The boxes in the lower half of Figure 1 show the percentage of normal hair cells remaining after the exposure as a function of the total cumulative number of hair cells measured from the basal end of the cochlea. Region and extent of absent or damaged hair cells are indicated by blackened areas. All three animals received an exposure of one hour duration. Cat LC 100 was exposed to a 135 dB SPL tone;* cats LC 76 and LC 110, to a 140 dB SPL tone.

As indicated in Figure 1, cat LC 100 suffered no hearing loss as a result of the exposure. Cats LC 76 and LC 110 had hearing losses in the frequency range 500 to 12000 Hz, with maximum loss occurring at the exposure frequency. At 4000 Hz, there was a permanent hearing loss of approximately 60 dB.

Figure 1 also shows that cat LC 100 sustained essentially no hair cell destruction as a function of the exposure. Only slight damage is indicated along the outer (3rd) row of outer hair cells (OHCs). Cats LC 76 and LC 110 had severe but narrow lesions in all three rows of OHCs and in the single row of inner hair cells (IHCs); lesions occurred in the upper basal turn about midway along the length of the cochlea.

Exposure to 2000 Hz

The results of exposure to a 2000 Hz tone that was one hour in duration are shown for three animals in Figure 2.

Cat RC 101 was exposed to a 130 dB SPL tone; it sustained a hearing loss in the range 1000 to 12000 Hz, with a maximum loss of about 38 dB occurring at the frequency of the exposure tone. No hearing loss was found for either the very low or very high frequencies. Cat LC 73, exposed to a 135 dB SPL tone, had greater hearing loss than RC 101 for all frequencies from 500 Hz to 12000 Hz. Again, the maximum loss, about 58 dB, occurred at the frequency of the exposure tone. Cat LC 67 was exposed to a 140 dB SPL tone; it had complete loss of hearing for frequencies from 2000 to 12000 Hz, severe loss at 1000 Hz, and smaller losses at 500 and 250 Hz.

The anatomical results of the exposures to a 2 kHz tone are given in the lower half of Figure 2. Cat RC 101, which had a 38 dB hearing loss at 2000 Hz, had only minor hair cell damage. With the exception of a very narrow lesion in which approximately 40% of the OHCs in the first row were missing, cat RC 101 appeared to have a nearly normal cochlea. Cat LC 73, which had a 58 dB hearing loss at 2000 Hz, had severe destruction of both inner and outer hair cells; the greatest damage to the outer hair cells occurred in two regions toward the apical end of the cochlea. One region of severe damage was in the approximate location of maximal activity caused by a 2000 Hz tone; the second region was at the apical tip of the cochlea and is apparently not reflected in the audiometric data. It should be noted that the lesion that occurred more distant from the apical end is much like the lesions found in cats LC 76 and LC 110 as a result of exposures to a 4 kHz tone; the lesion is confined to a very limited region of the basilar membrane.

The anatomical results of cat LC 67, which suffered the greatest PTS as a result of the 2000 Hz exposure, are also shown in Figure 2. As expected from the audiometric data, cat LC 67 had complete destruction of IHCs and all three rows of OHCs in all but the apical tip of the cochlea.

Exposure to 1000 Hz

The audiometric results for six animals exposed to a 1 kHz tone for one hour are shown in Figure 3. Exposure levels were 120, 130, and 140 dB. Cat LC 115 was exposed to a 120 dB SPL tone and had no PTS. Cat RC 97, exposed to a 130 dB

SPL tone, had a moderate amount of PTS at all frequencies above 250 Hz with the maximum loss again occurring at 2000 Hz. Cats LC 91 and LC 89 were both exposed to a 140 dB SPL tone and, after exposure, failed to respond to any signals between 250 and 20000 Hz. No audiometric tests were made at 125 Hz.

The anatomical results for the four animals exposed to 1000 Hz are shown in Figure 3. Cat LC 115 had essentially no damage as a result of the exposure. Cat RC 97 had a double-peaked lesion of the outer hair cells and a single narrow region of damage to inner hair cells. Cats LC 91 and LC 89 both suffered total destruction of OHCs and IHCs throughout all but the apical end of the cochlea.

Exposure to 125 Hz

The audiometric results for six animals exposed at 125 Hz for four hours are shown in Figure 4. Cat LC 96 was exposed to a 150 dB SPL tone and suffered no PTS. Cats LC 71 and LC 77 were exposed to a 155 dB SPL tone and, like LC 96, had normal post-exposure audiograms. Cat LC 105, exposed to a 157.5 dB SPL tone, had severe PTS for frequencies from 125 to 16000 Hz, the amount of PTS being greater at the high frequencies. Cats LC 88 and LC 70, which were exposed to intensities of 152.5 dB SPL and 155 dB SPL, respectively, failed to respond, after exposure, to tones throughout the range 125 to 16000 Hz.

The anatomical results for the animals exposed to 125 Hz are also shown in Figure 4. Cats LC 77, LC 96, and LC 71 suffered little or no damage from the sound exposure. Cat LC 105 had

complete loss of hair cells in the basal half of the cochlea and partial loss of cells in the upper turns. Cat LC 88 had almost total loss of OHCs and extensive damage to IHCs. Cat LC 70 had a nearly normal cochlea; limited damage occurred to outer hair cells at the apical end of the cochlea and to a small percentage of inner hair cells near the apex. There was also loss of OHCs in row 3 near the round window.

DISCUSSION

Locus of damage--frequency of exposure-tone

The relationship between frequency of exposure and locus of inner ear damage is most evident in those animals in which the exposure caused only a narrow region of damage. As a result of exposure at 4 kHz, for example, animals LC 76 and LC 110 sustained narrow but severe lesions of both OHCs and IHCs in the upper basal turn of the cochlea. In cases in which the exposure frequency was lowered to either 2000 Hz (LC 73) or 1000 Hz (RC 97), however, the region of hair-cell loss occurred at locations further from the basal end of the cochlea (approximately in the middle turn). In animals that suffered severe hair-cell destruction after exposure to either 2000 Hz or 1000 Hz (LC 67, LC 89, LC 91), all hair cells were destroyed from the upper middle or lower apical turn to the apical end of the cochlea.

Exposure to 125 Hz, in all but one case, produced either no damage (LC 77, LC 96, LC 81) or widespread destruction of hair cells (LC 105, LC 88) throughout the cochlea, extending from the basal turn to the apical end.

Inner ear damage - exposure level

For frequencies of 4,000, 2,000, and 1,000 Hz, the relationship between the amount of inner ear damage and the sound pressure level of the exposure tone is clearly seen: the extent of damage was, without exception, greater in those animals that received the highest exposure levels.

There was greater variability of results for exposure to 125 Hz; nevertheless, the two animals with greatest inner-ear damage were exposed to sound pressure levels as high or higher than the exposure levels of animals with less inner ear damage.

More interesting, perhaps, was the small amount of change in the exposure-intensity required to alter the resultant cochlear damage from minimal to severe. At all exposure frequencies investigated, for example, a 10 dB increase in the intensity of the exposure meant the difference between moderate or non-existant and severe destruction in the cochlea. At 125 Hz, a change of less than 5 dB in the exposure intensity sometimes meant the difference between a normal and a destroyed cochlea.

Hearing loss - exposure level

For exposures to 1000, 2000, and 4000 Hz, hearing loss increased with increase in sound pressure level. Again, there was greater variability in results for animals exposed to 125 Hz.

Hearing loss - region and extent of inner-ear damage

For animals exposed at 1000, 2000, and 4000 Hz, the frequency at which maximal hearing loss occurred corresponded well with the locus of hair-cell damage. Often, however, the range of frequencies for which hearing loss occurred did not correspond well with the width of the cochlear lesion (particularly with narrow

cochlear lesions). Animals with maximal hearing loss at 4000 Hz (LC 76 and LC 110), for example, had damage to both IHCs and OHCs in the upper basal turn of the cochlea. Animals with maximal hearing loss at 2 kHz (RC 101 and LC 73) had hair cells destroyed in the middle turn of the cochlea (although LC 73 had a second lesion near the apex). The animal with maximal hearing loss at 1 kHz (RC 97) also had lesions in the middle and apical turns. In each of these cases, the range of frequencies at which hearing loss occurred was greater than would have been expected based on the width of the cochlear lesion or lesions. There are several possible explanations of this discrepancy. The most likely is that the histological data presented above are based primarily on a determina- of the apparent "presence" or "absence" of each hair cell using phase-contrast microscopy. No further attempt was made to ascertain the condition of a hair cell. More subtle alterations of a hair cell than its elimination or severe distortion would not be seen although other techniques such as electron microscopy might have revealed damage sufficient to render the cell non-functional. This explanation would also account for such discrepancies as represented by RC 101 which had considerable hearing loss but only minimal hair cell damage. The lesions sustained at the apical end of the cochleas of LC 73 and RC 97 may have been produced by interruption of the blood supply to the apical region, a disorder that might be unrelated to the sound exposure (see Bernstein and Schuknecht, 1967).

In all cases in which the exposure caused widespread destruction of OHCs and IHCs (LC 105, LC 88, LC 91, LC 89, LC 67),

there was a corresponding severe loss of hearing at all frequencies or complete loss at higher frequencies and severe loss extending into the lower frequency-range. In one case, LC 70, the post-exposure hearing loss cannot be explained in terms of inner-ear damage.

Hearing loss - destruction of IHCs or OHCs?

The data obtained in this experiment do not permit any definitive answer to the question: Does the audiogram reflect the condition of inner hair cells, outer hair cells, or both? Evidence from several animals are relevant to this question. Cat LC 88 had almost complete destruction of OHCs, but a large number of IHCs in the apical turn were still present; post-exposure testing indicated complete loss of hearing. LC 91 also had complete loss of hearing although some IHCs remained in the apical and basal turns. RC 101 had post-exposure hearing loss for frequencies from 2000 to 10,000 Hz although IHCs remained throughout the cochlea.

The above cases (LC 88, LC 91, and RC 101) might be taken as evidence that the audiogram does not necessarily reflect the condition of the IHCs.

In contrast to the above cases, LC 73 had normal hearing for low frequencies despite the nearly total destruction of OHCs in the apical turn; IHCs were intact in the apical turn and throughout the cochlea except for a narrow region in the middle turn. In this case, it might be argued that the audiogram does reflect the condition of the IHCs.

Finally, in considering the results of the present study and of many others that have related hearing loss to inner-ear damage,

the limitations of methods used in assessing inner-ear damage must be kept in mind. No single histological technique provides a complete assessment of intracochlear damage or change resulting from an exposure. It is necessary to accurately assess damage to hair cells, intracochlear changes such as mechanical destruction of nerve fibers, disruption of the synaptic region between sensory cell and nerve fiber, interruption of blood supply, and damage to supporting structures.

There are also limitations in the methods used to measure changes in hearing in experimental animals. Occasional anomolous results may be expected. In an animal with a severe hearing loss, it may be difficult to obtain threshold measurements. For a normal animal or one with moderate hearing loss, tests of absolute threshold may be started by presenting a tonal signal well above threshold and decreasing it until the animal no longer makes a behavioral response. For animals with severe hearing loss, it may not be possible to use a tonal stimulus that is much above threshold. Therefore, great care must be taken not to produce "neurotic" behavior by creating highly stressful conditions for an animal--particularly one that has been exposed to loud sound or has otherwise been treated so as to produce a severe hearing deficit. The presence of tinnitus may also disrupt the animals performance in response to weak tonal signals.

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SUMMARY

Experimental animals (cats) were exposed to tones of 125, 1000, 2000, and 4000 Hz at sound pressure levels in the range 120 to 157.5 dB, and for durations of one hour (1000, 2000, 4000 Hz) or four hours (125 Hz). Pure tone audiograms were obtained for each animal before and after exposure. Post-exposure tests were continued until complete recovery of hearing had occurred or until a stable permanent threshold shift had been measured. Cochleas of animals were examined by phase-constrast microscopy; condition of all hair cells was recorded. Extent of inner-ear damage and range of frequencies for which hearing loss occurred increased as exposure tone was decreased in frequency. For example, exposure to 4000 Hz produced damage in a restricted region of the cochlea and hearing loss for a relatively narrow range of frequencies; exposure to 125 Hz produced wide-spread inner ear damage and hearing loss throughout the frequency range 125 to 6000 Hz.

Figure 1. Pure tone thresholds (upper half of figure) and plots of inner ear damage (lower half of figure) for animals exposed to 4000 Hz tone. For each animal, the O-dB line represents its preoperative hearing level. Hearing loss or permanent threshold shift (PTS) is plotted as a deviation from the O-line.

In the plots of inner ear damage, the base line (labeled 0,4,8,12 etc) indicates the number of hair cells, as counted starting at the base of the cochlea. For example, 4 means that to that point, four hundred hair cells had been counted. The number of destroyed or damaged hair cells is shown by the black-shaded areas. For example, in the plot for Cat LC 110, all of the hair cells in outer row 3 were damaged in the region occupied by hair cells 1400 to about 1600 (upper basal turn). The damage was less in the other two rows of outer hair cells and in the single row of inner hair cells.

NR= No response to tone at maximum level available.

Figure 2. Pure-tone thresholds and inner ear damage of animals exposed to 2000 Hz (Figure 1 for explanation)

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Figure 3. Pure-tone thresholds and inner ear damage for animals exposed to 1000 Hz (Figure 1 for explanation)

Figure 4. Pure-tone thresholds and inner ear damage for animals exposed to 125 Hz. (Figure 1 for explanation.)

REFERENCES

Bernstein, J. M. and Schuknecht, H.F., 1967, Lesions of the apical region of the cochlea. J. Laryngol., Otol., 81, 1-26.

Bredberg, G. and Hunter-Duvar, I. Chapter Five, Behavioral Tests of Hearing and Inner Ear Damage, Handbook of Sensory Physiology, Volume 5 Auditory System. In press.

Dolan, T.R., Bredberg, G., Ades, H.W., and Neff, W.D., 1970, The Behavioral and Histological Effects of Auditory Over-exposure in the Cat. 80th Meeting, Acoustical Society of America. (abstract)

Kemp, E.H. 1935, A critical review of experiments on the problem of stimulation deafness. Psychol. Bull., 32, 325-342.

Olson, R.E. and Ludwig, H.A. 1967, Solid-state electronic switch for auditory research. IEE Trans., Bio-Med. Engl. 12, 193-195.